

PROJECT

Asthma

12/20/01

**Experimental plans for asthma study:****Purpose**

Evaluate the efficacy of anti-C5 (BB5.1) on asthma mouse model.

**Animals**

BALB/c mice (8-12 weeks old) are purchased from the Jackson laboratory and maintained at Alexion's pathogen free facility.

**Antigen sensitization and challenge**

BALB/c mice will be sensitized on day 1 and 14 by intraperitoneal injection of 20ug OVA, (Grade V, Sigma) emulsified in 2mg aluminum hydroxide(Alum imject, Pierce) in a total volume of 100 ul. All mice will be challenged daily with OVA for 10minutes via the airways (1% OVA in saline) for 3 days (day 28,29, and 30), using ultrasonic nebulization. In day 32, mice will be provoked with OVA (5% in saline) for 10 minutes to elicit an antigen-induced early-phase reaction (EPR) and late-phase reaction (LPR). Negative control mice will be sensitized with alum only and challenged with saline instead of OVA.

**1 Plan one:****(1) prophylactic treatment**

Beginning on day 25 the experimental groups will be injected with either anti-C5 (40mg/kg, S.C. injection in 0.2 ml saline) or Dex(2mg/kg, S.C. injection in 0.2 ml saline) also day 29 and 31. After 5 hours of 5% OVA provocation, mice will be sacrificed and bronchoalveolar lavage (BAL) will be performed by instilling of 1ml ice-cold PBS through the tracheal cannula, followed by gentle aspiration.

The BAL fluid will be collected.

1. Cell differential counts.
2. IgE measurement.

**3. Analysis of cytokines Cell differential counts. IL-4,IL-5,IL-13 and TGF-beta**

After BAL, lungs will be inflated with 10% buffered formalin(1ml), removed from chest cavity and fixed in 10% formalin at least 24h than stained with hematoxylin and eosin and examined by light microscopy for evaluation of the severity of inflammation.

**(2) therapeutic treatment**

In day 35, mice will be provoked with 5% OVA again, than the mice will be injected with anti-C5 (40mg/kg, I.V. injection in 0.2 ml saline) or Dex(2mg/kg, I.V. injection in 0.2 ml saline). After 5 hours, the mice will be done at both BAL and histology as the same as above.

**2 Plan two:**

In day 35, Baseline lung resistance and methacholine induced AHR will be measured in OVA sensitised mice and non-sensitised mice. By increasing doses of intravenous methacholine (5,15,45,137,411ug/kg), we will make invasive measurement of tracheal pressure in 5-min interval (see detail in our invasive method). Mice can be treated with BB5.1 in a dose of 40mg/kg 24 hours, 8 hours, 4 hours, and 2 hours before methacholine challenge. Control group will treated with 135.8 and PBS.

Read and Understood By

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Signed

3/12/02

Date

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9/25/02

Date